Journal of Chromatography B, 1025 (2016) 110–118

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Simultaneous measurement of etravirine, maraviroc and raltegravir in pigtail macaque plasma, vaginal secretions and vaginal tissue using a LC–MS/MS assay

Anna K. Blakney[a], Yonghou Jiang[a], Dale Whittington[b,**], Kim A. Woodrow[a,*]

[a] Department of Bioengineering, University of Washington, Seattle, WA, USA
[b] Department of Medicinal Chemistry, Mass Spectrometry Center, University of Washington, Seattle, WA, USA

ARTICLE INFO

Article history:
Received 19 February 2016
Received in revised form 21 April 2016
Accepted 30 April 2016
Available online 2 May 2016

Keywords:
Etravirine
Maraviroc
Raltegravir
Plasma
Vaginal tissue
Vaginal secretions

ABSTRACT

Etravirine (ETR), maraviroc (MVC) and raltegravir (RAL) are promising antiretroviral drugs being used in HIV treatment and may be interesting for prevention applications such as oral or topical pre-exposure prophylaxis. Here we describe a sensitive and accurate method for the simultaneous detection of ETR, MVC and RAL from pigtail macaque plasma, vaginal secretions, and vaginal tissue. This method is characterized by a straightforward precipitation extraction method, a limit of quantification <0.5 ng mL^{-1} for all three antiretrovirals bolstered by a corresponding internal standard for each drug analyte, and short run time. Quantification is performed using positive ion electrospray triple quadrupole mass spectrometry. This method was validated over clinically relevant ranges for the three ARV drugs in all three matrices: 0.1–100 ng mL^{-1} for ETR, 0.05–100 ng mL^{-1} for MVC and 1–100 ng mL^{-1} for RAL. Our method is accurate and precise, with measured mean inter-assay precision (%CV) and accuracy (% bias) of 5.08% and 1.96%, respectively, while the mean intra-assay precision and accuracy were 3.44% and 1.08%. The overall post-extraction recovery for ETR, MVC and RAL was >94% in all cases. We also show that extracted biological samples are stable after storage at room temperature or 4 °C and after three freeze/thaw cycles. This is the first analytical method capable of quantifying ETR, MVC and RAL in biological matrices relevant for pre-clinical testing of oral or topical HIV prevention methods in pigtailed macaques.

Published by Elsevier B.V.

1. Introduction

Disparate rates of HIV acquisition between men and women have motivated development of oral and topical prevention methods intended as discreet, woman-initiated dosage forms. Poor performance of the 1% tenofovir gel in recent clinical trials has spurred the inclusion of new, more potent antiretrovirals into microbicides [1]. Furthermore, much like formulations for treatment of HIV, cocktails of multiple antiretrovirals with varied mechanisms of action may prove to have synergistic capacity and lower required drug dosage, with a lower risk for development of resistant HIV strains [2]. The majority of HIV prevention dosage forms in development, including pills [1], gels [3], rings [4], films [5] and fibers [6], aim to prevent acquisition of HIV in the genital tract [7]. HIV prevention formulations are typically tested first in non-human primate models for safety and efficacy, motivating development of quick, highly sensitive bioanalytical methods for the detection of antiretroviral cocktails in affected tissues, including vaginal tissue and secretions, as well as plasma.

Etravirine (TMC125, Intelence®) is non-nucleoside reverse transcriptase inhibitor (NNRTI) that is highly active against both wild-type and NNRTI-resistant HIV strains [8], has a higher genetic barrier to the development of resistance than currently available NNRTIs [9], and has been shown to be safe, tolerable and effective in the treatment-experienced patients [10]. Maraviroc (UK-427,857, Celsentri®, Selzentry®) is a selective CCR-5 antagonist with potent anti-HIV activity [11], that has been investigated for microbicide use alone in gel form [12] and in combination with dapivirine as a vaginal ring [13]. Raltegravir (MK-0518, Isentress®) is a novel HIV-1 integrase inhibitor that prevent proviral DNA-strand transfer [14],

Abbreviations: CAN, acetonitrile; ARV, antiretroviral; ETR, etravirine; HIV, human immunodeficiency virus; LC–MS/MS, liquid chromatography tandem mass spectrometry; MeOH, methanol; NNRTI, non-nucleoside reverse transcriptase inhibitor; MVC, maraviroc; RAL, raltegravir.

* Corresponding author at: 3720 15th Ave Ne, Box 355061, Seattle, WA 98195, USA.
** Corresponding author at: 1959 NE Pacific St., HSB 1-093, Seattle, WA 98195, USA.
E-mail addresses: dalewhit@uw.edu (D. Whittington), woodrow@uw.edu (K.A. Woodrow).

http://dx.doi.org/10.1016/j.jchromb.2016.04.048
1570-0232/Published by Elsevier B.V.

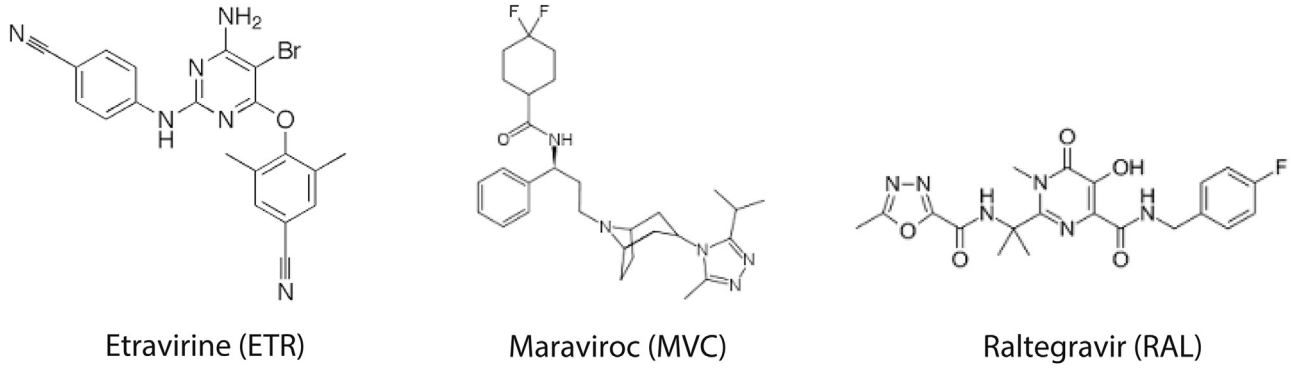

Fig 1. Chemical structures of antiretroviral drugs.

which has been shown to protect macaques from SHIV challenge when applied post-exposure in a topical gel [15].

To date, several methods have been published for the quantification of ETR, MVC and/or RAL in a variety of tissue matrices. Fayet et al. validated a LC–MS/MS assay for ETR, MVC and RAL, in addition to darunavir, in human plasma [16]. Other LC–MS/MS assays have been published for quantification of each drug alone, including ETR in human plasma and peripheral blood mononuclear cells and rat plasma [17,18], MVC in human plasma, urine and cerebrospinal fluid [19], and RAL in human plasma and peripheral blood mononuclear cells [20,21]. The combination of ETR, MVC and RAL has also been quantified using LC–MS/MS in human plasma in addition to a combination of eight other relevant antiretroviral drugs [22]. Currently, no LC–MS/MS assay has been validated to quantify ETR, MVC and RAL combinations in vaginal tissue and secretions, or plasma.

Here we present a validated analytical method for the simultaneous quantification of etravirine, maraviroc and raltegravir in pigtail macaque vaginal tissue, vaginal secretions and plasma using liquid chromatography coupled with tandem triple quadrupole mass spectrometry detection. A single step extraction is performed using acetonitrile precipitation of plasma, vaginal secretions and homogenized vaginal tissue, after the addition of ETR-$^{13}C_6$, MVC-d_6 and RAL-d_6 as internal standards. The mixture is vortexed vigorously, centrifuged for 10 min at 10,000 RPM and filtered before a 2 μL injection into an EMD Chromolith® Performance RP-18e 100–3 mm analytical column. Analytes are separated using a gradient method of water with 10 mM formic acid and 1:1 acetonitrile:methanol with 10 mM formic acid. Our method enables the simultaneous quantification of these three antiretroviral drugs for pharmacokinetic and pharmacodynamic preclinical studies in pigtail macaque studies.

2. Materials & methods

2.1. Reagents and tissues

Etravirine (ETR) and maraviroc (MVC) drug standards were obtained from the NIH AIDS Reagent Program (Division of AIDS, NIAID, NIH, Bethesda, MD, USA). Raltegravir (RAL) drug standard and maraviroc-d_6 (MVC-d_6), raltegravir-d_6 (RAL-d_6), and etravirine-$^{13}C_6$ (ETR-$^{13}C_6$) internal standards were purchased from Alsachim, Inc. (Illkirch-Graffenstaden, France). Acetonitrile (ACN), methanol (MeOH) and formic acid, all Optima-LC/MS grade, were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Ultrapure water was obtained using a Milli-Q UF-Plus apparatus (Millipore, Burlington, MA, USA). Naïve pigtail macaque (macaca nemestrina) plasma, vaginal secretions and vaginal tissue were purchased from the Washington National Primate Research Center (WaNPRC) Tissue Distribution Program (Seattle, WA, USA).

2.2. Instrumentation and equipment

The liquid chromatography system was composed of an I-Class Acquity UPLC (Waters Corporation, Milford, MA, USA) with a direct infusion syringe pump and temperature-controlled 96 vial autosampler maintained at 4 °C. Samples were separated on a Chromolith Performance RP-18e 100–3 mm analytical column (Merck, Darmstadt, Germany) at ambient conditions. The chromatographic system was coupled to a Waters Xevo TQ-S tandem quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA) with a Micromass Zspray™ Atmospheric Pressure Ionization (API) Source. The LC–MS/MS system and data analysis was carried out using MassLynx® software (version 4.1) (Waters Corporation, Milford, MA, USA).

Processing of plasma, secretions and tissue was performed using a Precellys® 24 tissue homogenizer (Bertin Corporation, Rockville, MD, USA) (tissue only) and a Sorvall Legend 14 centrifuge (Thermo Scientific, Waltham, MA, USA).

2.3. Calibration standard, internal standard and quality control (QCs) solutions

Stock solutions of ETR, MVC and RAL (Fig. 1) were prepared at a concentration of 1 mg mL^{-1} in DMSO, and then were combined and diluted with ACN to make working solutions at concentrations of 10,000 ng mL^{-1}, 200 ng mL^{-1} and 2 ng mL^{-1} (Table 1). Calibration standards were then diluted with appropriate amounts of ACN (neat standards), or plasma, vaginal secretions, or tissue to achieve a working calibration range from 0.01–100 ng mL^{-1}. Internal standards (ETR-$^{13}C_6$, MVC-d_6, RAL-d_6) were likewise diluted as stock solutions in DMSO at 1 mg mL-1. A working solution of all three internal standards was prepared at a concentration of

Table 1
Concentrations of stock solutions, working solutions and QC controls.

Solution	Stock Solution Solvent	Stock Solution Concentration	Working Solution Concentration[a]	Calibration Range	QC Controls
Drug (ETR, MVC, RAL)	DMSO	1 mg mL^{-1}	10,000; 200; 2 ng mL^{-1}	0.01-100 ng mL^{-1}	0.5; 5; 50 ng mL^{-1}
Internal Standard(ETR-$^{13}C_6$, MVC-d_6, RAL-d_6)	DMSO	1 mg mL^{-1}	10,000 ng mL^{-1}; 10 ng mL^{-1}	N/A	N/A

[a] Obtained by dilution of stock solution with ACN.